TECHNICAL NOTE

THE WATER-ASSOCIATING PROPERTIES OF COMMINUTED COWHIDE CORIUM*

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ABSTRACT

To obtain data on the water-associating properties of collagen, water was removed from comminuted cowhide corium and from reconstituted calfskin collagen in two steps: by centrifugation and by drying in a vacuum oven; and the ratio of removed water to dry collagen in each of these steps (labile and bound water, respectively) was compared with the total water (gross water). Heated (gelatinous) corium held more bound water than unheated (fibrous) corium; but unlike fibrous corium, it showed no labile water until gross water was raised to 80 percent, and then it associated increasing amounts as gross water was increased.

Collagen is known to be a very hygroscopic material which associates with water in many different ways, and through the interaction of its many polar groups with water it displays surface-active properties. In fact, it has been shown by many experimenters that water stabilizes collagen at levels of molecular organization from secondary to quarternary, and possibly even higher (1).

Many studies *via* sorption isotherms of collagen in contact with water in the vapor phase have been carried out, but there is a paucity of quantitative data dealing with collagen in contact with liquid water, which is the case most frequently encountered in collagen applications. Consequently, experiments were performed in which water was removed from collagen samples in a two-stage process consisting of centrifugation to remove loosely associated (labile) water and drying in a vacuum oven to remove tightly associated (bound) water. These values were then compared with the total (gross water) present in the original sample. Measurements were made on fibrous samples of native cowhide corium and fibrous reconstituted calfskin collagen. They were then repeated on these materials after they were gelatinized by heating to 80°C. for 15 minutes. This aspect is important because the thermal transitions that take place alter the number of exposed reactive groups on the collagen molecule.

The cowhide corium was purchased from a commercial tannery in the form of flesh splits from limed hides which had been carefully washed. When subjected to Differential Thermal Analysis, these samples showed characteristic transition temperatures of 63° and 210°C. This material was comminuted to granules and short fibers in a high speed cutter set for a 0.060 in. cut, in accordance with the techniques of Elias *et al.* (2), and comminution was carried out in a mixture of 0.1 percent propionic and 0.05 percent benzoic acid to yield a slurry of pH 5.5, a pH that persisted throughout the experiments. At this stage the milled cowhide corium had a gross water content of 70 percent, or 2.33 g. of water per g. of dry corium. A portion of this was freeze-dried and the product had a gross water content of four percent.

The reconstituted calfskin collagen was obtained by solubilization of corium at 10°C. in citrate buffer using automated equipment which maintained pH 3.4 and an ionic strength of 0.44 (3), followed by dialysis to exhaustion of electrolyte at the same temperature.

Water was added to the gelatinized corium to give a series of samples whose gross water content increased in steps from 2.33 g. of water per g. of dry collagen to 19.0 g. of water per g. of dry collagen to see if a mass action law was operating. Attempts to add water to the unheated corium, in expectation of raising its gross water content resulted in the creation of two distinct phases rather than a smooth slurry. Consequently, the unheated samples were run at only 2.33 g. of gross water per g. of dry collagen. Water was also added to portions of the reconstituted collagen to give a series of unheated (fibrous) samples having a gross water content of 2.33 g. per g. of dry collagen and a series of heated (gelatinized) samples having gross water contents ranging from 2.33 to 19.0 g. per g. of dry collagen. In this way, the gross water contents of the reconstituted samples correspond to those of the comminuted corium.

The gross sample of moist collagenous material was spun in a Spinco Model L‡ centrifuge equipped with a No. 30 rotor at 30,000 R.P.M. for two hours to remove labile water and yield a net sample. The net sample was then dried to constant weight in a vacuum oven at 50°C., which required about 18 hours. This removed water more closely associated with the collagen, which is designated bound water. The residue was considered dry collagen.

The two-stage water removal process removed equal amounts of labile and bound water from the fibrous cowhide corium, an indication that water is associated with collagen in more than one way. Some of it is so loosely coupled it can be removed by centrifugation; a further portion can be removed by heating under vacuum; and it is very likely that some water is so tightly associated that it resists these methods of separation.

Figure 1 shows the results when this process was applied to comminuted cow-

‡Reference to brand or firm name does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

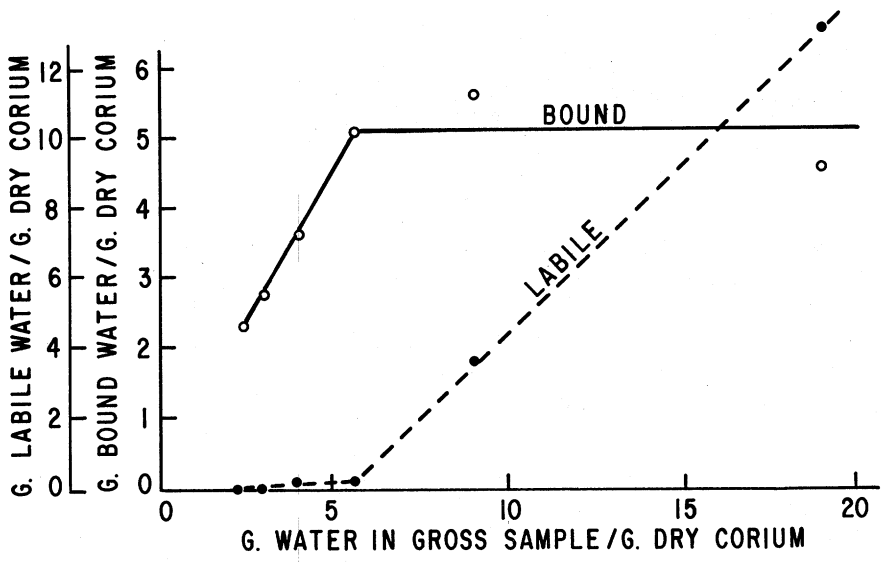

FIGURE 1.—Association of water with cowhide corium.

hide corium that had been gelatinized by heating to 80°C. for 15 minutes. The value of 5.7 g. of gross water per g. of dry collagen now appears to be a critical point. Up to this value labile water remains practically nil while bound water increases linearly. Beyond this point labile water increases linearly, while bound water remains constant at a value of about 5 g. per g. of dry collagen. This indicates that, for the lower values of gross water, gelatinized corium associates more bound water at the expense of labile water than fibrous comminuted corium does, and gelatinization shifts water to a more securely coupled state. Since infrared studies (4, 5) indicate that an important mode of binding water to collagen is hydrogen-bonding between adjacent peptide chains, gelatinization in breaking these bonds would leave the newly exposed carbonyl oxygen and amide hydrogen atoms free to enter into further hydrogen bonded coupling with water.

Prior to subjecting reconstituted fibrous collagen to the two-stage separation process, gross water was added to bring it to the same value as in the runs with fibrous comminuted corium (2.33 g. of water per g. of dry collagen). Separation then yielded 1.53 g. of labile water and 0.80 g. of bound water per g. of dry collagen, *i.e.,* more labile water than comminuted corium, but less bound water. This could mean that in the case of fibrous reconstituted collagen, sites with binding potential are functioning as interfibrillar cross-links, giving reconstituted collagen a more rigid structure.

Gelatinized reconstituted collagen showed water-binding characteristics in close accord with gelatinized corium, which means that while reconstituted collagen

has a more cross-linked structure, it yields to gelatinization to the same extent as comminuted cowhide corium.

When these experiments were repeated with freeze dried cowhide corium, the results were the same as in the case of fresh material.

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